between voltage- and ligand-dependent pathways. Development of well-defined model-independent metrics of interaction energies will be crucial for delineating molecular interaction pathways and understand the mechanisms of voltage-transduction.

**1219-Symp**
**Structural Investigation of a Bacterial Voltage-Gated Sodium Channel $Na_vRh$**
**Nieng Yan**, Xu Zhang.
Tsinghua University, Beijing, China.
Voltage-gated sodium ($Na_v$) channels are essential for the rapid depolarization of nerve and muscle, and are important drug targets. Elucidation of the structures and functional mechanisms of $Na_v$ channels will shed light on fundamental ion channel mechanisms and facilitate potential clinical applications. A family of bacterial $Na_v$ channels, exemplified by NaChBac ($Na^+$-selective Channel of Bacteria), provides a good model system for structure-function analysis. We determined the crystal structure of $Na_vRh$, a NaChBac orthologue from marine bacteria, *Rickettsiales sp. HIMB114* (denoted Rh), at 3.05 Å resolution. The channel comprises an asymmetric tetramer. The carbonyl oxygen atoms of Thr178 and Leu179 constitute an inner site within the selectivity filter ($_{178}$TLSSWE$_{183}$) where a hydrated $Ca^{2+}$ can bind and resides in the crystal structure. The outer mouth of the $Na^+$ selectivity filter, defined by Ser181 and Glu183, is closed, as is the activation gate at the intracellular side of the pore. The voltage sensors adopt a depolarized conformation with all the gating charges exposing to the extracellular side. We hypothesize that $Na_vRh$ is captured in an inactivated conformation. Comparison of $Na_vRh$ with $Na_vAb$ reveals that the VSD segments undergo discordant conformational shifts concurrent with domain rotation that may underlie the electromechanical coupling mechanism of voltage-gated channels.

**1220-Symp**
**Voltage-Sensor Domain Proteins: Phosphoinositide Signal, Proton Permeation and Molecular Tools**
**Yasushi Okamura, PhD, MD**.
Physiology, Osaka University, Suita, Japan.
Voltage-sensor domain (VSD) is the key transmembrane module for sensing membrane voltage in voltage-gated ion channels and voltage-sensing phosphatase. Hv1 (also called VSOP) mainly expressed in phagocytes and human sperm consists only of VSD and the coiled-coil region, operating as the voltage-gated proton channel. In Hv1, VSD plays dual roles of pH-dependent voltage sensing and proton-selective permeation. These properties are innate to protomer. However, dimerization of Hv1 enables cooperative voltage-dependent gating. The voltage-sensing phosphatase, VSP, is the voltage-activated phosphoinositide phosphatase in which single VSD is tightly coupled with the phosphatase with significant homology to the phosphatase and tensin homolog deleted on chromosome 10 (PTEN). Phosphoinositides such as PIP3 and PI(4,5)P2 are dephosphorylated by VSP upon membrane depolarization. Physiological functions of VSP are largely unknown but fibroblasts heterologously expressing VSP exhibit neurite-like fine processes, raising a possibility that VSP may play role in regulating cell shape by phosphoinositide turnover. VSP itself is a useful tool to rapidly manipulate phosphoinositide level by simple membrane depolarization to study roles of phosphoinositides that play roles in diverse biological events. VSD of voltage sensor domain proteins could also be utilized as a molecular material to devise voltage probe for visualizing electrical activities in cells and tissues. I will talk about recent findings of molecular mechanisms of VSP and Hv1 and examples of molecular tools based on VSP.

**1221-Symp**
**Structural Basis of Voltage-Dependent Gating in Ci-VSP**
**Eduardo Perozo**, Qufei Li, Sherry Wanderlin.
Dept of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, USA.
The transduction of electric fields into protein motion plays an essential role in the generation and propagation of cellular signals. Voltage-sensing domains (VSD) carry out these functions through reorientations of discrete gating charges in the S4 helix. The voltage sensitive phosphatase from C. intestinalis (Ci-VSP) is controlled by a standard VSD with high sequence similarity to the S1-S4 segments found in $Na^+$ and $K^+$ channels. To address some of the fundamental questions regarding how membrane proteins sense transmembrane voltages, we have pursued structural and biophysical information on Ci-VSP's isolated VSD, under conditions that stabilize the Up and the Down conformations. These will be discussed in the context of explicit mechanisms for voltage sensing.

# Platform: Calcium Signaling

**1222-Plat**
**Reliable Encoding of Stimulus Intensities by Random Sequences of $Ca^{2+}$ Spikes**
Kevin Thurley, Stephen C. Tovey, Gregor Moenke, Victoria L. Prince, Abha Meena, Andrew P. Thomas, Alexander Skupin, Colin W. Taylor, **Martin Falcke**.
Max Delbrück Center, Berlin, Germany.
$Ca^{2+}$ is a ubiquitous intracellular messenger. Extracellular stimuli often evoke sequences of $Ca^{2+}$ spikes, and it is suggested that spike frequency may encode stimulus intensity. However, the timing of spikes is random because each interspike interval (ISI) has a large stochastic component. We also find that average ISI vary considerably between cells. Can individual cells reliably encode stimuli when $Ca^{2+}$ spikes are so unpredictable? Analysis of $Ca^{2+}$ spikes evoked by receptors that stimulate formation of inositol 1,4,5-trisphosphate ($IP_3$) reveals that signal-to-noise ratios are improved by slow recovery from global feedback inhibition and that they are robust against perturbations of the signalling pathway. Despite variability in the frequency of $Ca^{2+}$ spikes between cells, steps in stimulus intensity cause the stochastic period of the ISI to change by the same factor in all cells. These fold-changes reliably encode changes in stimulus intensity, and they entail an exponential dependence of average ISI on stimulation strength. We find the latter to apply to different cells, stimuli and stimulation intensities. We conclude that $Ca^{2+}$ spikes allow reliable signalling in many cells despite randomness and cell-to-cell variability because global feedback robustly reduces noise, and stimulation steps are encoded by fold-changes in the stochastic period of the ISI.

**1223-Plat**
**Coupling of Chemical and Mechanical Sensing in Fibroblast Cells**
**Josephine Lembong**[1], Bo Sun[2], Matthew Rogers[3], Howard A. Stone[4].
[1]Chemical and Biological Engineering, Princeton University, Princeton, NJ, USA, [2]Physics, Oregon State University, Corvallis, OR, USA, [3]Firmenich Inc., Plainsboro, NJ, USA, [4]Mechanical and Aerospace Engineering, Princeton University, Princeton, NJ, USA.
Cells constantly sense their local chemical environment and make decisions based upon the information received. This chemosensing process, although stochastic on the individual cell level, exhibits highly regulated responses in multicellular organisms. Two key features, intercellular communication and first-responder cells, define this process. To understand the collective behavior induced by these two factors, we culture fibroblast cells inside a PDMS-on-glass channel, to which we deliver ATP solution, and study their calcium dynamics in response to the chemical stimulation. We demonstrate the existence of first-responder cells and how the presence of gap junctions influences the first step of the collective response, in that there are correlations in the response of neighboring cells. In addition, we investigate the effect of mechanical environment on a colony's response by studying cell responses when encapsulated in thin hydrogel films. By comparing the results from when the cells are cultured on glass to hydrogel-embedded cells, where intercellular communication is only possible via diffusing molecules, we find that (i) persistent calcium oscillations of individual cells occur only for cells embedded in hydrogel, and (ii) a colony of hydrogel-embedded cells show no synchronization. The fraction of a colony that responds to ATP increases with hydrogel elasticity and ATP concentration. For those cells that oscillate, we find that at high ATP concentration (>40 μM) cells inside a stiffer gel have fewer oscillations than those inside a softer gel. The distributions of oscillation periods have modes that are decreasing with increasing ATP concentration. Our observations and measurements highlight the role of mechanical environment for influencing spatial and temporal dynamics in cell colonies and tissues.

**1224-Plat**
**$Na^+$ Channels Control Metabolism and Global $Ca^{2+}$ Signaling by Inducing $Na^+$ and $Ca^{2+}$ Responses that are Propagating into the Mitochondria of Beta Cells**
**Israel Sekler**.
Physiology, Ben Gurion University, Beer-Sheva, Israel.
$Na^+$ channels in β cells are abundantly expressed and prone to prolong activation, yet their role in regulating cellular $Na^+$ fluxes or mitochondrial $Ca^{2+}$ transient, thereby metabolism is poorly understood Here, we combined fluorescent ion and electrophysiological analysis with molecular control of transporter expression and mitochondrial metabolism in MIN6 and primary beta cells. Glucose induced a TTX sensitive cytosolic $Na^+$ and $Ca^{2+}$ response that were propagating into the mitochondria. Mitochondrial $Ca^{2+}$ influx was largely blocked in cell transfected with siRNA of the mitochondrial $Ca^{2+}$ uniporter MCU. Knockdown of the mitochondrial $Na^+$/ $Ca^{2+}$ exchanger (NCLX) and $Na^+$ dose response analysis show that NCLX is major mitochondrial $Na^+$ influx pathway, tuned to sense cytosolic $Na^+$ changes mediated by high glucose. TTX sensitive mitochondrial $Ca^{2+}$